

REDUCED NUMBERS OF RETRIEVED OOCYTES IN SMOKING WOMEN

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Abstract

The results of a three-year study concerned with different ovarian responses to hormonal stimulation in 220 smoking and non-smoking female patients involved in an IVF programme are described. The smoking women, as compared with the never-smokers, had a lower response to hormonal stimulation, i.e., a significantly lower mean number of 14 mm follicles (10.3 vs. 14.2) and a lower mean number of retrieved oocytes (7.3 vs. 11.9). The ratio of the mean number of oocytes retrieved from smokers to that from never-smokers was 0.614; this corresponded to the mean reduction (38.6%) in oocyte numbers in the smokers. The smoking women had a significantly lower fertilisation rate compared to the never-smokers and to those who stopped smoking: 49.8 %, 67.2 % and 64.8 %, respectively. A significant, negative correlation was found between age and the number of retrieved oocytes in the smokers, as compared with the never – smokers.

Key words

smoking, oocytes, fertilisation rate

INTRODUCTION

Many epidemiological studies have demonstrated the effect of smoking on a delay in conception by approximately 2 months in smokers, as compared with never-smokers (1, 2, 3). Methods of assisted reproduction allow us to study relationships between smoking and the number of retrieved oocytes and their ability to be fertilised. These methods ‘therefore’ can contribute to an understanding of the mechanisms of action of smoking on female fertility.

We present our results of a three-year study concerned with different responses to hormonal stimulation in smoking and non-smoking patients involved in an IVF programme.

MATERIALS AND METHODS

A total of 220 female patients from the infertile couples treated by *in vitro* fertilisation and embryo transfer (IVF/ET) at the Centre for Assisted Reproduction were included in this study during the period from October 1997 to December 1999. Complete results of all the factors examined and a written informed consent from every patient were prerequisites for participation in the study.

In each patient, medical history was obtained, including smoking habits verified by the measurement of urine cotinine both at the start and at the end of hormonal stimulation. Women were identified as smokers, former smokers and never smokers. Women with an average consumption of 0 to 1 cigarette per day were regarded as occasional smokers, women with an average consumption of 11 to 20 cigarettes per day were regarded as moderate smokers, and women with an average consumption exceeding 20 cigarettes per day were regarded as heavy smokers.

Follicle development was stimulated with gonadotropines (hMG, FSH), at a dose of 150–225 IU per day, combined with GnRH analogues in the short (buserelin) or long (triptorelin) protocols. The stimulated cycle was monitored by assessment of 17-beta estradiole, LH, progesterone and by ultrasound folliculometry. After the appropriate ovarian response had been obtained, 5,000 to 10,000 IU of human chorion gonadotropine (hCG) were administered to induce oocyte maturation. After 34 to 36 hours, oocytes were retrieved by means of transvaginal ultrasound guided puncture and aspiration. The fertilisation of oocytes was confirmed 18 to 24 h after *in vitro* insemination by detection of two pronuclei. The number of fertilised oocytes was expressed as the fertilisation rate (FR, %)

For statistical evaluation, the *t*-test in the EPI INFO programme was used.

RESULTS

Among 220 patients, the following distribution of smoking habits was found: 56 women (25.5 %) were current smokers, 40 women (18.2 %) were former smokers and 124 (56.3 %) were never – smokers. Out of 56 current smokers, 22 patients (39.3 %) were occasional smokers, 23 (41.1 %) smoked up to 10 cigarettes daily and 11 (19.6 %) were moderate smokers with 11 to 20 cigarettes per day. This detailed characteristic of smoking habits was not convenient for statistical analysis and ‘therefore’ only smoker and never-smoker categories were eventually used in the tables. The average age of the whole group was 29.4 to 4.3 years, with no differences between the smokers and never-smokers.

The smoking women, as compared with the never-smokers, had a lower response to hormonal stimulation, i.e., a significantly lower mean number of 14 mm follicles (10.3 vs. 14.2) and a lower mean number of retrieved oocytes (7.3 vs. 11.9 (*Table 1*). The ratio of the mean number of oocytes retrieved from the smokers to that from the never-smokers was 0.614; this corresponded to the mean reduction in numbers of oocytes (38.6 %) in the smokers.

The smoking women had a significantly lower fertilisation rate (49.8 %), as compared to the never-smokers (67.2 %) and to those who stopped smoking (64.8 %) (*Table 2*). A significant, negative correlation between age and the number of retrieved oocytes was found in the smokers, as compared with the never smokers (*Table 3*).

Table 1
Response to hormonal stimulation in never-smokers (n = 124), former smokers (n = 40) and current smokers (n = 56).

	Whole group	Never-smokers	Former smokers	Smokers	<i>P</i> <
No. of follicles	12.0	14.2	14.1	10.3*	0.05
S.D.	4.8	4.9	5.1	4.2	–
No. of oocytes	10.3	11.9	7.9	7.3	N.S.
S.D.	0.4	0.6	0.6	0.5	–

SD, standard deviation; NS, non-significant

Table 2
Fertilisation rate in women with different smoking habits

	Whole group (n = 220)	Never-smokers (n = 124)	Former smokers (n = 40)	Smokers (n = 56)	<i>p</i> <
Mean FR (%)	62.3	67.2	64.8	49.8 *	0.01
S.D.	36.1	33.2	35.2	40.3	–

FR, fertilisation rate, i.e., % of fertilised oocytes; SD, standard deviation.

Table 3
Age-related differences in the mean number of oocytes retrieved from women with different smoking habits

Age (years)	Never- smokers		Former smokers		Smokers	
	Mean	95 % CI	Mean	95 % CI	Mean	95 % CI
20–24	11.9	6.2–14.4	7.4	3.4–18.4	8.9	1.4–14.1
25–29	14.1	9.4–18.5	10.7	4.5–16.9	12.1	9.1–13.6
30–34	11.6	8.1–14.2	8.1	4.8–13.2	4.8*	1.2–8.1
35–40	9.9	4.8–14.6	5.3	5.3–7.5	3.1*	0.2–3.8

CI, confidence interval; *, *P* < 0.05

DISCUSSION

Zenzes (4) has summarised data from ten studies in which the numbers of oocytes retrieved from smoking and non-smoking women were reported. In all but one (5) of the published studies, the average number of oocytes was higher in never-smokers than in smokers. The ratio of the mean number of oocytes obtained from smokers and to the mean number of oocytes from never-smokers ranged between 0.559 and 0.980.

The weighted mean ratio for these nine studies was 0.920, which corresponds to a mean reduction in oocyte number by 8.0 % in smoking women. In moderate and heavy smokers, who could be identified in three studies (6, 7, 8), the weighted mean ratio was 0.828. This corresponds to a mean reduction in oocyte number by 17.2 %.

The ratio of the mean oocyte number in smokers to that in never-smokers in our study (0.614) is the second lowest one in comparison with the data reported above. This observation cannot be explained by the influence of age-related physiological decline in reproductive functions, as the women in our study had the lowest average age: 29.4 years as compared with 31.5 to 37.8 years in the other studies.

The influence of other factors related to different lifestyles, such as diet with low content of ascorbic acid, preference to nutrients high in saturated fatty acids and higher caffeine consumption may have contributed to the reduction in oocyte numbers observed in smokers. Although we did not ask the women involved in the IVF programme about their alcohol consumption, we cannot exclude a higher alcohol intake by smokers, since we found it in another study from the same region (9).

The physiological, age-related decline in numbers of retrieved oocytes was found in the smoking women but not in the non-smoking women in our study. As the age of smokers was correlated with the duration of the smoking period, we could confirm the dose-response effect of smoking. This indicates that the synergistic effects of age and smoking may accelerate the rate of oocyte destruction (10).

The increased sensitivity of oocytes to damage shortly after they resume meiosis is considered to be a possible mechanism of smoking influence on oogenesis. This hypothesis is supported by studies concerned with the assessment of oocyte maturity stage; the results of these studies show; an increasing proportion of mature oocytes in smokers; as compared to never-smokers, and a concurrent proportional deficiency of intermediary oocytes (8). There were significant, positive correlations to increasing cotinine levels measured in follicular fluid.

Mature oocytes have better chances of fertilisation than immature oocytes and thus the fertilisation rate (numbers of embryos/oocytes) may be used in evaluation

of maturity status. Higher rates of fertilisation in smokers, compared with never-smokers, were reported in several studies (7, 10, 11, 12, 13) but other studies did not confirm these findings (14, 15). Our results are similar to the latter group since the smokers had a significantly reduced fertilisation rate in comparison with the never-smokers (49.8 % vs. 67.2 %). These conflicting results indicate that the mechanism of smoking action is not simple (16) and that more attention should be given to this issue.

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SNÍŽENÝ POČET ZÍSKANÝCH OOCYTŮ U KOUŘÍCÍCH ŽEN

S o u h r n

Jsou předkládány výsledky tříleté studie, zaměřené na sledování rozdílné odpovědi ovarí na hormonální stimulaci u kouřících a nekouřících žen v souboru 220 pacientek léčených v programu IVF. Kuřačky (56 žen) měly nižší úroveň odpovědi na hormonální stimulaci, projevující se ve významně nižším průměrném počtu vytvořených folikulů 14 mm (10,3 vs. 14,2) a získaných oocytů (7,3 vs. 11,9). Poměr průměrného počtu oocytů získaných od kuřaček a nekuřaček byl 0,614, což odpovídá průměrnému snížení počtu oocytů o 38,6 %. Kuřačky měly rovněž významně nižší fertilizační poměr v porovnání s nekuřačkami a bývalými kuřačkami: 39,8 % versus 57,2 %, resp. 54,8 %. U kuřaček byla rovněž nalezena negativní korelace mezi věkem pacientek a počtem získaných oocytů.

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